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COMPLETE SPECIFICATION.

Purine Derivatives and their Preparation.

We, THE WELLCOME FOUNDATION LIMITED, a British Company, of 183–193 Euston Road, London, N.W.1, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention in one aspect provides a new method for making intermediates in the manufacture of 6-mercapto-9-glycosylpurines and 2-amino-6-mercapto-9-glycosylpurines. These latter compounds interfere with cell division and inhibit unnatural growth.

Three methods for the preparation of 6-mercapto-9-glycosylpurines have been described in the literature. In the first, the silver salt of 6-benzylmercaptopurine was condensed with tetraacetylglucopyranosyl chloride, and the product deacetylated in ethanolic ammonia, and debenzylated with sodium in liquid ammonia, to give 6-mercapto-9-glucopyranosylpurine. In the second, the chloromercui derivative of 6-chloropurine was reacted with triacetylribofuranosyl chloride and the product deacetylated in methanolic ammonia to give 6-chloro-β-D-ribofuranosylpurine, which was reacted with sodium hydrogen sulphide in methanol to give 6-mercapto-9-β-D-ribofuranosylpurine. In the third, $2^1,3^1,5^1$-tri-*O*-benzoylinosine was reacted with phosphorus pentasulphide to give 6-mercapto-9-$2^1,3^1,5^1$-tri-*O*-benzoyl-β-D-ribofuranosylpurine, which was deacylated with sodium methoxide to give 6-mercapto-9-β-D-ribofuranosylpurine. 2-amino-6-mercapto-9-β-D-ribofuranosylpurine was similarly prepared from $2^1,3^1,5^1$-tri-*O*-benzoylguanosine.

The 6-chloro-9-(*O*-acylated glycosyl) purines may be prepared from 6-chloropurine. The 9-$2^1,3^1,5^1$-tri-*O*-acyl-β-D-ribofuranosyl derivatives, however, are much more satistorily prepared by the new method of this invention from a $2^1,3^1,5^1$-tri-*O*-acylinosine or a $2^1,3^1,5^1$-tri-*O*-acylguanosine by chlorination with a reagent such as phosphoryl chloride. This method, followed by thiation of the product, is found to be a more satisfactory route to the 6-mercapto-9-$2^1,3^1,5^1$-tri-*O*-acyl-β-D-ribofuranosylpurines than the direct thiation of the acylated inosine or guanosine with phosphorus pentasulphide, which results in excessive tar formation. The acyl groups are subsequently removed to give the 6-mercapto-9-β-D-ribofuranosylpurine or the 2-amino-6-mercapto-9-β-D-ribofuranosylpurine.

The 2-amino-6-mercapto-9-glycosylpurines are of special interest in chemotherapy, as the parent compound 2-amino-6-mercaptopurine has been found to be about twenty times more effective than 6-mercaptopurine in producing 50% inhibition of sarcoma 180 in the mouse.

This invention in another aspect provides as new compounds the 2-amino-6-chloro-9-$2^1,3^1,5^1$-tri-*O*-acyl-β-D-ribofuranosylpurines which are intermediates for making 2-amino-6-mercapto-9-β-D-ribofuranosylpurine.

The following examples illustrate the invention, whose scope is defined in the claims. All temperatures are given in degrees Celsius.

EXAMPLE 1.

100 ml. acetic anhydride was added to a suspension of 15 g. inosine in 200 ml. pyridine. The mixture was stirred until homogeneous, and kept on the steam-bath for 2 hours. The resulting clear amber-coloured solution was concentrated *in vacuo* to a semi-solid residue, which was dissolved with

[*Price 4s. 6d.*]

the evolution of heat by the addition of 250 ml. ice water. Upon cooling for 5 minutes in an ice-bath, a white crystalline solid appeared, which after an hour at 4° was filtered off and washed with ice water to give 19.5 g. $2^1,3^15^1$-tri-*O*-acetylinosine (89% yield), m.p. 230°. In absolute ethanol it had maximum ultra-violet absorption at 245 mμ.

100 ml. phosphoryl chloride was added to an intimate mixture of 16.0 g. $2^1,3^1,5^1$-tri-*O*-acetylinosine with 10 ml. dimethylaniline. The suspension was heated on the steam bath for 4 hours under anhydrous conditions. The homogeneous solution was then concentrated *in vacuo* on the steam bath to remove the excess phosphoryl chloride. The remaining brown syrup was dissolved in 250 ml. chloroform, and the solution was washed with 5 × 75 ml. water, when the washings were at *p*H 5—6. The chloroform layer was dried over anhydrous sodium sulphate and concentrated to a syrup. Final traces of chloroform were removed under high vacuum to give 14.8 g. crude 6-chloro-9-$2^1,3^1,5^1$-tri-*O*-acetyl-β-D-ribofuranosylpurine (89% yield) as a syrup. In water it had maximum ultra-violet absorption at 265 mμ.

Example 2.

25 ml. chloroform, followed by 10 g. benzoyl chloride, was added to a suspension of 5 g. inosine in 10 ml. pyridine cooled at 0° in an ice bath. The mixture was heated at 100° for 2 hours, allowing the chloroform to distil off. The resultant syrup was dissolved in 100 ml. chloroform. The solution was washed with saturated sodium bicarbonate solution and then washed with saturated sodium bisulphate solution followed by water. After drying over sodium sulphate, the chloroform solution was concentrated *in vacuo* to a syrup, which on treatment with anhydrous ether gave 10 g. $2^1,3^1,5^1$-tri-*O*-benzoylinosine (92% yield) as a solid. It analysed as N=9.61% (by theory N=9.68%), and in ethanol had ultra-violet absorption maxima at 230 and 265 mμ.

61 g. Phosphoryl chloride was added to a suspension of 5 g. $2^1,3^1,5^1$-tri-*O*-benzoylinosine in 5 ml. dimethylaniline, whereupon the solid dissolved. The mixture was heated at 100° for 4 hours. The resulting light yellow solution was poured into 800 ml. of an ice water-chloroform mixture (1:1 by volume). The water layer was extracted twice with chloroform. The chloroform layer was washed twice with saturated sodium bicarbonate solution and then with water. After drying over sodium sulphate, the chloroform layer was concentrated *in vacuo* to a syrup, which was dissolved in 100 ml. absolute ethanol and poured into 600 ml. water. Upon acidification to *p*H 2 with hydrochloric acid, 6 g. crude 6-chloro-9-$2^1,3^1,5^1$-tri-*O*-benzoyl-β-D-ribofuranosylpurine precipitated. It contained organic chlorine, gave a positive Molisch test, and in ethanol had ultra-violet absorption maxima at 230 and 260 mμ. The peak at 230 mμ is characteristic of the benzoyl derivatives while that at 260 mμ is similar to that of 6-chloropurine, which in aqueous solution has a peak at 265 mμ at *p*H 1.

Example 3.

250 ml. acetic anhydride was added to a suspension of 50 g. guanosine (commercial grade) in 500 ml. pyridine. The mxture was heated at 100° for 17 hours. The resulting solution was poured into 1600 ml. water and extracted with 3 × 200 ml. chloroform. The chloroform solution was washed with 2 × 200 ml. saturated sodium bisulphate solution and then with 2 × 200 ml. water, dried over sodium sulphate, and concentrated to a syrup *in vacuo*. The syrup on trituration with ether gave 63 g. crude solid $2^1,3^1,5^1$-tri-*O*-acetylguanosine. The product had maximum ultra-violet absorption at 238 mμ at *p*H 6 and at 265 mμ at *p*H 12—characteristics similar to those of guanosine (as would be expected).

240 g. phosphoryl chloride was added to a suspension of 25 g. $2^1,3^1,5^1$-tri-*O*-acetylguanosine in 35 ml. dimethylaniline. The mixture was heated at 100° under reflux for 4 hours. The resulting solution was concentrated *in vacuo* on the steam bath to a syrup, which was dissolved in 200 ml. chloroform. The solution was extracted with 75 ml. portions of water until the washings were at about *p*H 4. After drying over sodium sulphate, the chloroform layer was concentrated *in vacuo* to a syrup. Anhydrous ether was added. The resulting hygroscopic solid was dried *in vacuo* to give 12 g. 2-amino-6-chloro-9-$2^1,3^1,5^1$-tri-*O*-acetyl-β-D-ribofuranosylpurine. The product contained organic chlorine, gave a positive Molisch test, and in ethanol had ultra-violet absorption maxima at 252 and 285 mμ.

Example 4.

Guanosine was benzoylated by the method described in Example 2 for inosine. $2^1,3^1,5^1$-tri-*O*-benzoylguanosine was isolated as a crystalline solid, m.p. 205—208°, which analysed as N=11.36% (by theory N=11.75%), and in ethanol had ultra-violet absorption maxima at 230 and 270 mμ.

5 g. $2^1,3^1,5^1$-tri-*O*-benzoylguanosine was chlorinated with phosphoryl chloride by the method described in Examples 2 and 3. 2-amino-6-chloro-9-$2^1,3^1,5^1$-tri-*O*-benzoyl-β-D-ribofuranosylpurine was isolated as an amorphous powder, probably a phosphate salt. The product contained nitrogen, phosphorus, and organic chlorine, and analysed as N=9.57% (by theory, with 1 mole H_3PO_4, N=9.85%). In ethanol, it had ultra-

violet absorption maxima at 230 and 280 mμ.

WHAT WE CLAIM IS:—

1. A method for making a 6-chloro-9-2^1,3^1,5^1-tri-*O*-acyl-β-D-ribofuranosylpurine or a 2-amino-6-chloro-9-2^1,3^1,5^1-tri-*O*-acyl-β-D-ribofuranosylpurine characterised in that a 2^1,3^1,5^1-tri-*O*-acylinosine or a 2^1,3^1,5^1-tri-*O*-acylguanosine is treated with a chlorinating agent, such as phosphoryl chloride.

2. A method as claimed in Claim 1 characterised in the the *O*-acyl groups in the starting material are acetyl or benzoyl groups.

3. A method for the preparation of a 6-chloropurine derivative as described in any of the foregoing examples.

4. A 6-chloropurine derivative whenever prepared by the method of any of Claims 1 to 3 or by its obvious chemical equivalent.

5. A 2-amino-6-chloro-9-2^1,3^1,5^1-tri-*O*-acyl-β-D-ribofuranosylpurine.

6. 2-amino-6-chloro-9-2^1,3^1,5^1-tri-*O*-acetyl-β-D-ribofuranosylpurine.

7. 2-amino-6-chloro-9-2^1,3^1,5^1-tri-*O*-benzoyl-β-D-ribofuranosylpurine.

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